Wyoming Game & Fish Department
Wildlife Health Laboratory

Wyoming Game and Fish Department
2018 Brucellosis Surveillance in Non-Feedground Elk Herds
April 2019

Overview:

Each year the Wyoming Game and Fish Department (WGFD) monitors the distribution and prevalence of brucellosis within the state's elk populations by utilizing blood samples collected by hunters from their harvested animal. Approximately 10,000 blood collection kits are assembled and mailed to elk hunters successful in acquiring limited quota licenses within target surveillance areas. Surveillance is generally concentrated in elk herd units (HUs) of the Bighorn Mountains and herds that surround the Brucellosis Designated Surveillance Area (DSA) that do not use state or federal feedgrounds (see Figure 1). In addition, nearly a quarter of the all hunt areas (HAs) occurring outside of the DSA are surveyed each year, providing coverage of the entire brucellosis non-endemic area every 4-5 years.

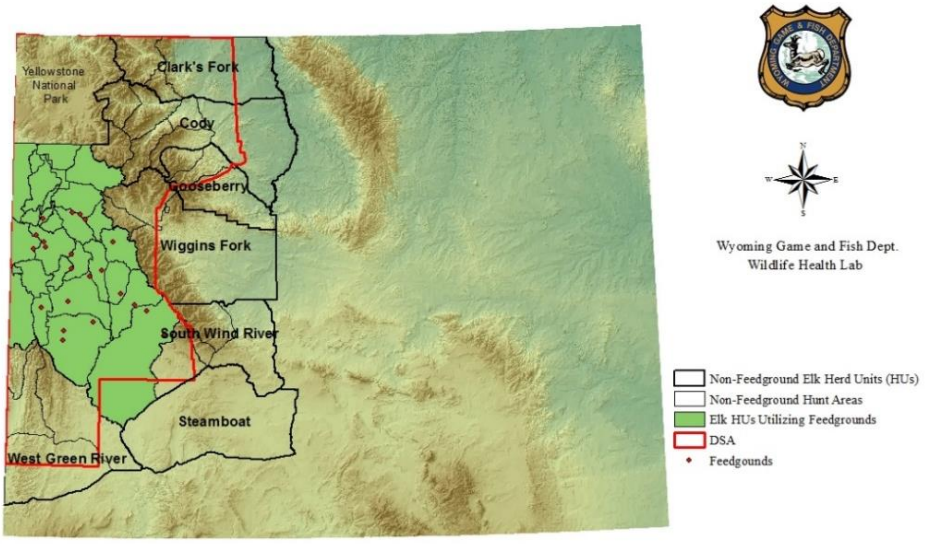

Figure 1. Locations of Wyoming feedgrounds, surrounding non-feedground elk herd units, and the Designated Surveillance Area (DSA)

The brucellosis surveillance program in non-feedground elk began in 1991, and over 17,000 blood samples have been analyzed for brucellosis since its inception. Brucellosis prevalence in the western portion of the state varies between 0-4% in the herd units south of the Greater Yellowstone Area (GYA) (i.e. South Wind River, and West Green River), and between 1-23% in the HUs east of the GYA (i.e. Clarks Fork, Gooseberry, Cody, and Wiggins Fork). In 2012, this disease was documented outside the GYA when it was discovered in elk of the northwestern Bighorn Mountains. Since the initial discovery, this disease has been sporadically detected in several hunt areas along the western slope of the Bighorn Mountains. Due to the lack of effective control measures to mitigate the spread of this disease, the documentation of seropositive elk outside of the GYA is alarming to both livestock and wildlife managers.

To better understand brucellosis in the Bighorn Mountains, a multi-year elk movement study was initiated in early 2016 to determine how this disease may have been introduced as well as to explore management implications should it become established. The study will examine movement and interactions of elk herds in the Bighorn Mountains as well as elk populations in the Bighorn Basin where seropositive animals have been previously documented. In addition, calving areas will be identified, as well as a predictive model on how brucellosis may further expand. Understanding the route of spread will enable development of management strategies that could minimize spread to neighboring elk herds as well as exposure to domestic cattle. Research elk that test seropositive for brucellosis are recaptured, euthanized, and tissues collected for culture and *Brucella* genomics.

2018 Surveillance:
The 2018 surveillance effort again concentrated on the Bighorn Mountains, particularly in HAs 39, 40, 41, and 49. The Cody Regional Wildlife Disease Biologist continued to focus on increasing blood sample returns from hunters, as well as implementing several measures to preserve blood samples prior to shipment to the laboratory. These efforts included a chance at a raffle for valuable hunting equipment for submitting a testable blood sample, and setting up multiple sample drop off points to prevent freezing of blood samples while in the mail. Efforts were also focused on those HAs on the DSA's southeastern border (HAs 25, 27 and 28) to ensure continued monitoring of the endemic/nonendemic border. Surveillance outside of the known brucellosis endemic area occurred in the central area of the State (see Figure 2).

The number of HAs surveyed and the number of blood collection kits mailed to hunters was based on the priorities of the WGFD and the Wyoming Livestock Board, while balancing the capacity of the WGFD Wildlife Health Laboratory (WHL). The 2018 surveillance effort was supported by the Department, and by a cooperative agreement with the Animal and Plant Health Inspection Service.

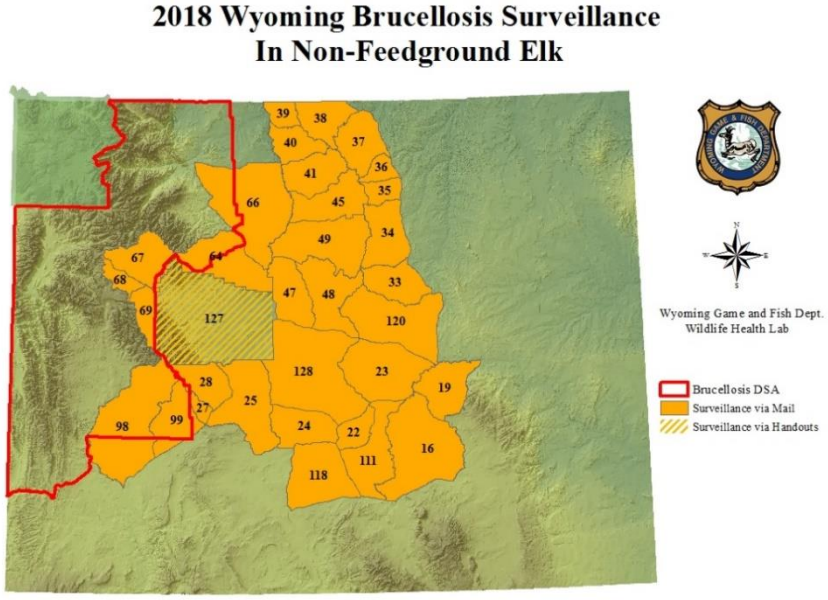

Figure 2. Elk HAs surveyed in 2018 for brucellosis in hunter-killed elk.

Methods:
In 2018, over 10,500 blood collection kits were mailed or directly handed to elk hunters successful in limited quota elk license drawings in the HAs targeted for surveillance. Kits consist of a 15 ml sterile polypropylene conical tube, a paper towel, an instruction/data sheet, as well as a prepaid mailing label for return shipping. Samples were also obtained opportunistically in association with various research efforts where animals were captured and bled for disease testing.

All useable serum samples were analyzed at the WHL. Serologic assays for exposure to *B. abortus* were conducted and interpreted using current National Veterinary Services Laboratories (NVSL) protocols for fluorescence polarization assay (FPA) in microplates and tubes. Serological profiles were categorized using the United States Department of Agriculture's brucellosis eradication uniform methods and rules for Cervidae (US Department of Agriculture-APHIS 91-45-16, 2003). The FPA plate test was used to screen all samples. Positive reactions on the plate assay were confirmed with the FPA tube test. Reactors originating outside of the known endemic area were submitted to NVSL for confirmation with the complement fixation test. Serologic data (seroprevalence levels) on elk within the known endemic area is based on yearling and adult females, but males and juveniles are included in surveillance data outside of the known endemic area. Including serologic data from males and juveniles offers improved detection of brucellosis in areas where this disease is not known to occur.

Nearly all serum samples received in 2018 (98%) were tested for exposure to *B. abortus*. This was a departure from previous years, where only the transparent serum samples were retained and tested. As serologic tests have improved and become less subjective, most hemolyzed serum samples are now suitable for testing and can contribute to surveillance data. Research by Jessica Jennings-Gaines of the WHL found that titers remained detectable even at 100% hemolysis, but those individual titers varied depending on the degree of hemolysis (Jessica Jennings-Gaines, unpublished data). Serum samples were only discarded if FPA results varied more than 15 points between duplicate runs on the same assay, and could not be confirmed upon re-test. Samples that had less than 15-point variation, but could not be confirmed were submitted to NVSL for testing and classification. Research continues on FPA testing of hemolyzed samples and a final report is expected in the coming year.

Results and Discussion:

A total of 1,559 elk blood samples were received by the WHL in 2018, with 1,529 those being suitable for testing. 830 useable samples were collected from the Bighorn Mountains; 176 of those were from yearlings or adult cows harvested in hunt areas where seropositive elk had been previously documented (see Figure 3). No seropositive elk were documented in the herd units that comprise the Bighorns. Unfortunately, attempts to obtain a *B. abortus* isolate through culture of lymph nodes collected from hunter killed or movement study animals have thus far been unsuccessful.

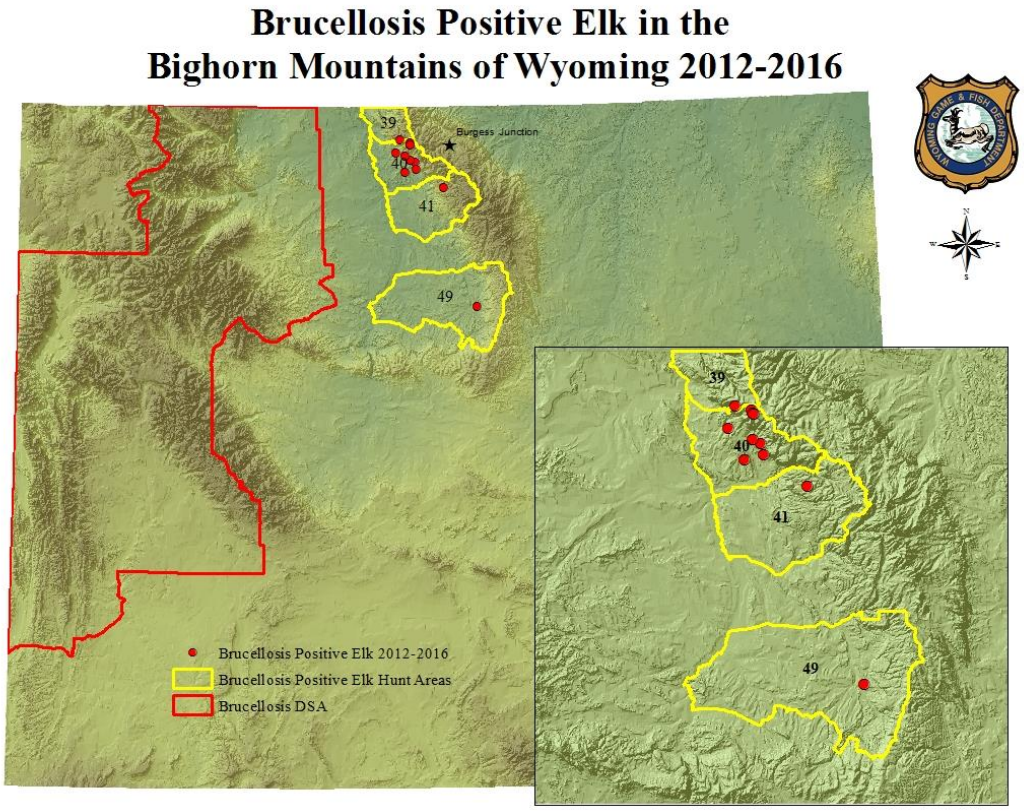

Figure 3. Locations of seropositive elk in the Bighorn Mountains; no new positives were identified in 2017 or 2018.

Table 1 outlines the number of samples analyzed in each of the HAs in the Bighorn Mountains as well as the associated HU. The 95% confidence interval is also listed for each HA and HU in Table 1. This value is calculated from the total samples collected from 2012 to 2018 and provides 95% certainty that the prevalence of brucellosis within that HA/HU falls within the specified range (see 95% confidence lower and upper columns), not the given prevalence determined for a particular year.

Table 1. Total useable blood samples tested from elk harvested in the Bighorn Mountains along with the 95% confidence interval of seroprevalence based on total samples 2012 to 2018.

		2018			Total Samples 2012-2018			95% Confidence (2012-18)	
Elk Hunt Area / Herd Unit (HU)	**Age/Sex**	**Samples**	**Positive**	**Prevalence**	**Samples**	**Positive**	**Prevalence**	**Lower**	**Upper**
33	All	25	0	0.0%	131	0	0.0%	0.0%	2.8%
34	All	48	0	0.0%	210	0	0.0%	0.0%	1.7%
47	All	14	0	0.0%	56	0	0.0%	0.0%	6.4%
48	All	53	0	0.0%	195	0	0.0%	0.0%	1.9%
49	All	93	0	0.0%	367	1	0.3%	0.0%	1.5%
	Cows	44	0	0.0%	125	0	0.0%	0.0%	2.9%
120	All	29	0	0.0%	146	0	0.0%	0.0%	2.5%
Total South Bighorn HU	All	262	0	0.0%	1105	1	0.1%	0.0%	0.5%
	Cows	44	0	0.0%	125	0	0.0%	0.0%	2.9%
35	All	45	0	0.0%	181	0	0.0%	0.0%	2.0%
36	All	16	0	0.0%	89	0	0.0%	0.0%	4.1%
37	All	47	0	0.0%	181	0	0.0%	0.0%	2.0%
38	All	104	0	0.0%	642	0	0.0%	0.0%	0.6%
39	All	62	0	0.0%	267	1	0.4%	0.0%	2.1%
	Cows	22	0	0.0%	136	0	0.0%	0.0%	2.7%
40	All	89	0	0.0%	454	8	1.8%	0.8%	3.4%
	Cows	47	0	0.0%	250	7	2.8%	1.1%	5.7%
Total North Bighorn HU	All	363	0	0.0%	1814	9	0.5%	0.2%	0.9%
	Cows	69	0	0.0%	386	7	1.8%	0.7%	3.7%
41	All	102	0	0.0%	551	1	0.2%	0.0%	1.0%
	Cows	63	0	0.0%	322	0	0.0%	0.0%	1.1%
45	All	103	0	0.0%	450	0	0.0%	0.0%	0.8%
Total Medicine Lodge HU	All	205	0	0.0%	1001	1	0.1%	0.0%	0.6%
	Cows	63	0	0.0%	322	0	0.0%	0.0%	1.1%
Total Bighorns	All	830	0	0.0%	3920	11	0.3%	0.1%	0.5%
	Cows	176	0	0.0%	833	7	0.8%	0.3%	1.7%

Brucellosis surveillance in the combined northern HUs (Clark's Fork, Cody, Gooseberry, and Wiggins Fork) of the DSA (see Figure 4) reported relatively stable seroprevalence over the past five years (13.9%; n=826 samples) compared to the previous five-year average of 14.2% (n=1,346) (see Figure 5). In 2018, 72 suitable samples were received from elk cows harvested from the South Wind River HU in the southern DSA, and 8 samples were received from the West Green River HU. Over the past five years, a total of only 113 samples have been analyzed from these HUs, with no seropositive animals identified (see Figure 5).

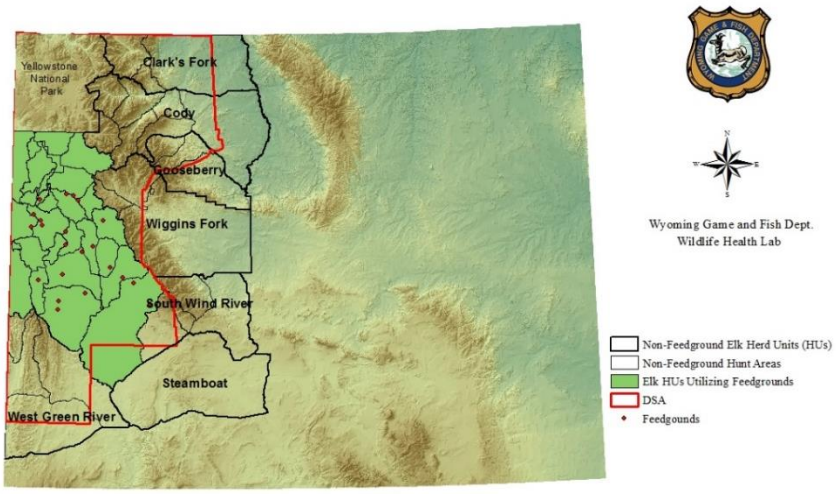

Figure 4. Locations of Wyoming feedgrounds, surrounding non-feedground elk herd units, and the Designated Surveillance Area (DSA).

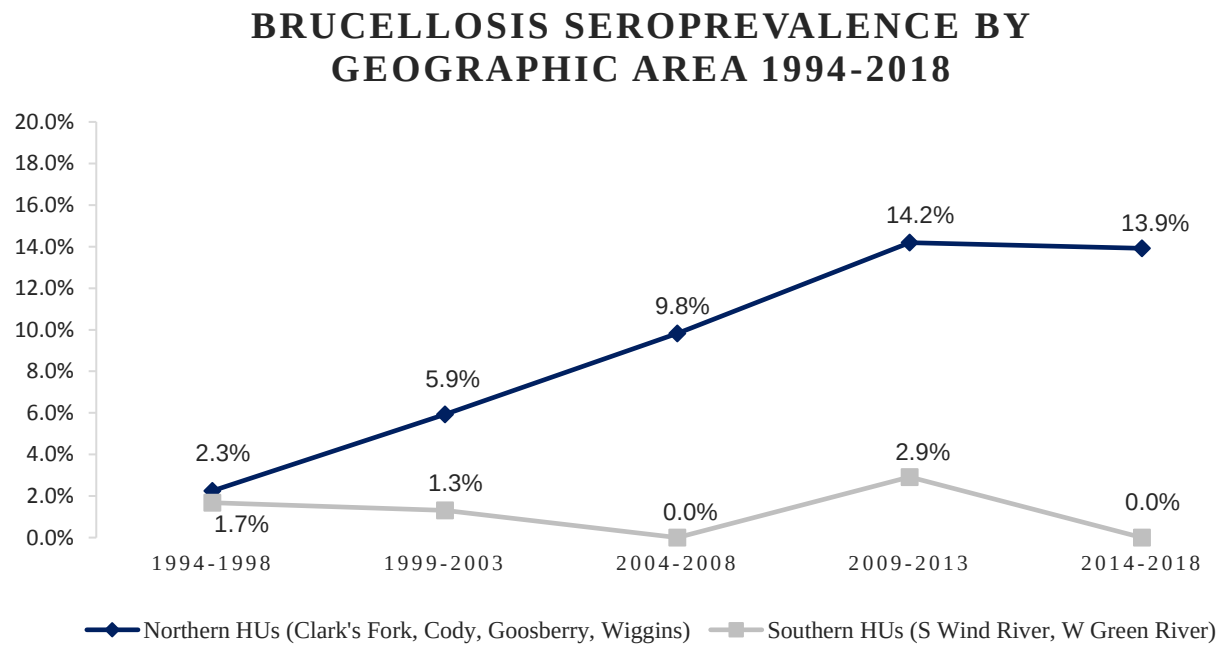

Figure 5. Seroprevalence through time in cow elk from northern and southern elk herd units (HUs) surrounding the DSA.

The five-year average seroprevalence varied considerably between the four northern HUs (see Figure 6). Brucellosis seroprevalence in the Gooseberry HUs has averaged 19.8% over the past fifteen years (n=612), with stable five-year averages ranging from 17.8% in the period from 2009-2013 (n=314) to 22.3% in the most recent period from 2014-2018 (n=229). Seroprevalence in the Cody HU has averaged 13.1% over the last ten years (n=1153), after increasing from 7.9% in the period from 2004-2008 (n=189). Sample sizes obtained from the Wiggins Fork (n=78 from 2014-2018) and Clarks Fork (n=80 from 2014-2018) HUs from 2004 through 2018 are insufficient to accurately estimate prevalence, and it is important to note that in most hunt areas, the sample sizes achieved through our annual surveillance are insufficient to estimate prevalence with good precision. Therefore, prevalence figures are combined into five-year totals to improve sample size and allow for statistical analysis.

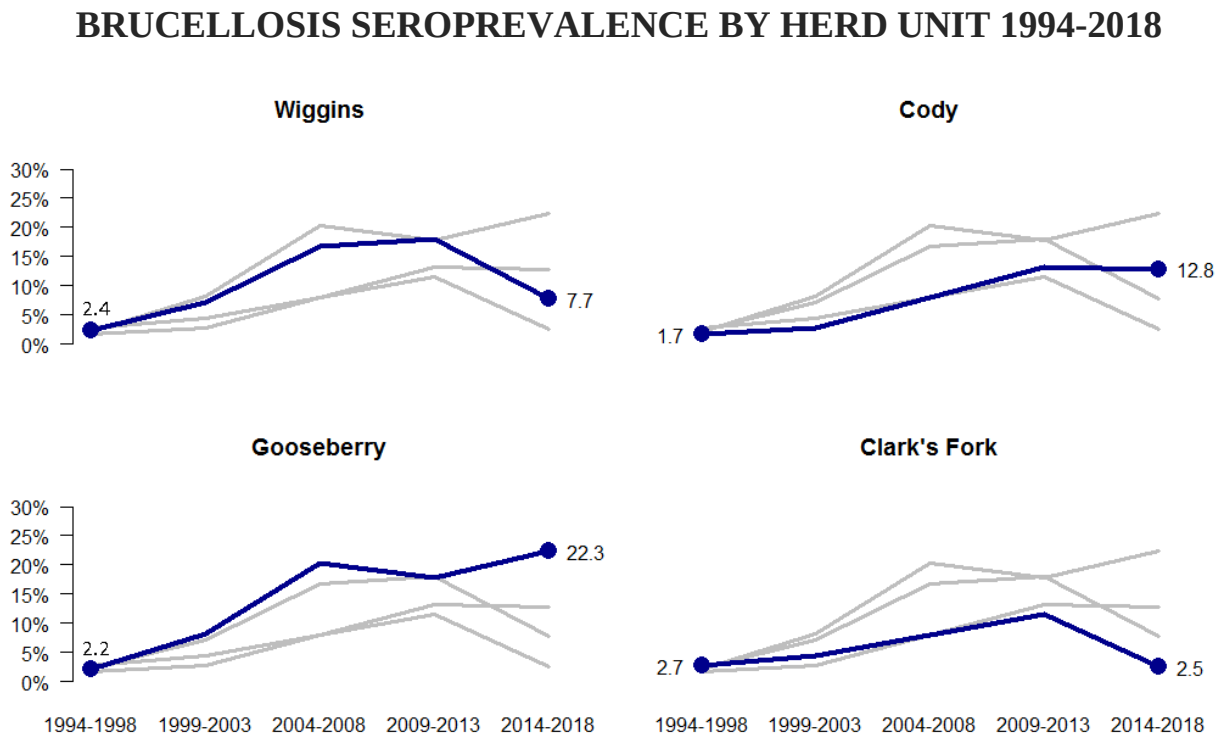

Figure 6. Brucellosis prevalence in cow elk in the Clark's Fork, Cody, Gooseberry and Wiggins elk herd units.

Brucellosis seroprevalence is also monitored within individual elk hunt areas of the DSA. Over the past twenty-five years, seroprevalence has gradually increased in hunt areas 58-59 and 61-63. In the last five years, the combined seroprevalence in these areas has averaged 19.8% (*n*=415). Many of the subpopulations in these hunt areas have been examined to determine if the increase in seroprevalence can be attributed to increasing elk density. Research found that the rates of increase were positively related to both large and small groups at high density, as well as larger groups at low densities (Brennan et al., 2014). In addition, these authors note that disease management strategies aimed at reducing population density or group sizes are unlikely to reduce transmission of the disease. Continued monitoring of all HAs along the southeastern slope of the Absaroka Range is warranted, as well as exploration of management actions that affect the prevalence of brucellosis in these populations.

Another goal of the 2018 surveillance was to increase surveillance along the southeastern border of the DSA where brucellosis positive elk have been previously identified, and which has not been extensively surveyed in the past five years. A combined fifty-seven samples were received from hunt areas 25, 27 and 28 in 2018, with no seropositive animals identified. These areas will remain a focus of the surveillance effort in 2019.

A total of 135 useable samples were collected in 2018 from the rotating surveillance program target areas (HAs 16, 19, 22-24, 111, 118 and 128). All samples tested negative for exposure to *B. abortus* on serological tests. In the past 28 years, 5,815 samples from the non-endemic area have been analyzed. To date, this disease has not been documented outside of western half of the state (see Figure 7).

The return rate of blood samples from hunters successful in harvesting an elk has remained relatively constant over the past five years with around 1,500 hunters submitting blood samples for analysis every year. On a per hunter basis, approximately 32% of successful hunters that received a blood collection kit voluntarily collected and submitted a sample for surveillance.

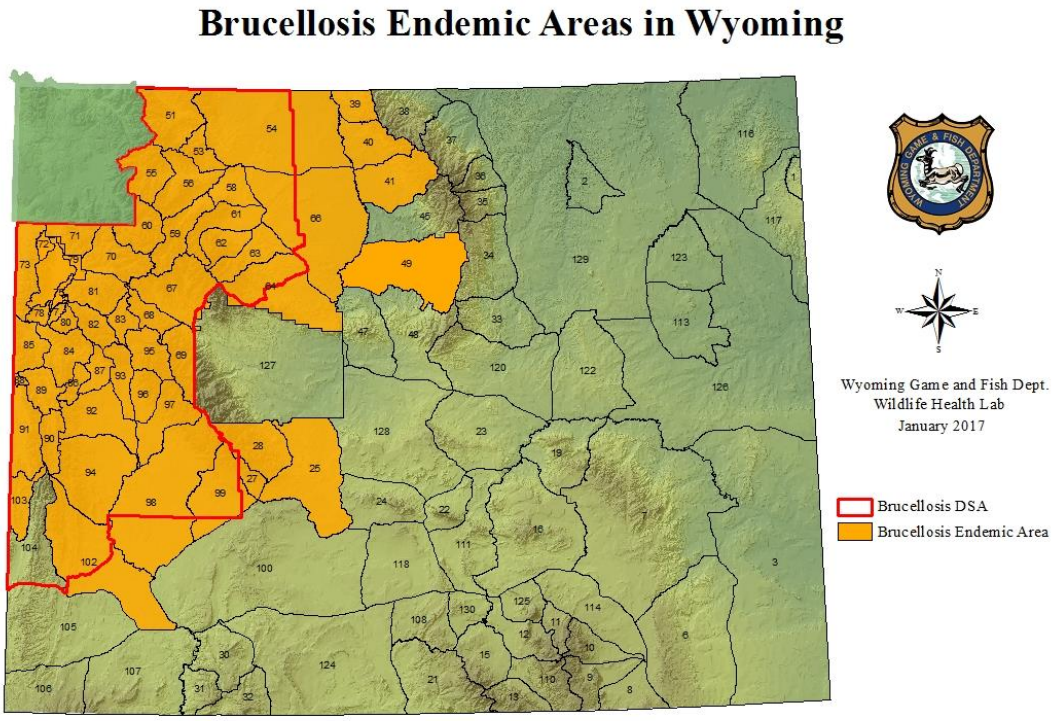

Figure 7. Brucellosis Endemic Elk Hunt Areas in Wyoming.

Brucellosis surveillance for 2019 will again concentrate on the Bighorn mountains as well as those hunt areas that surround the eastern DSA border (see Figure 8). Surveillance within the DSA will shift to the Cody HU. Statewide surveillance normally alternates through the elk hunt areas in the southern and eastern portions of the state, and in 2019, this effort will be directed to the southwestern area of the State (elk hunt areas 30, 31, 32, 106, 107, and 124).

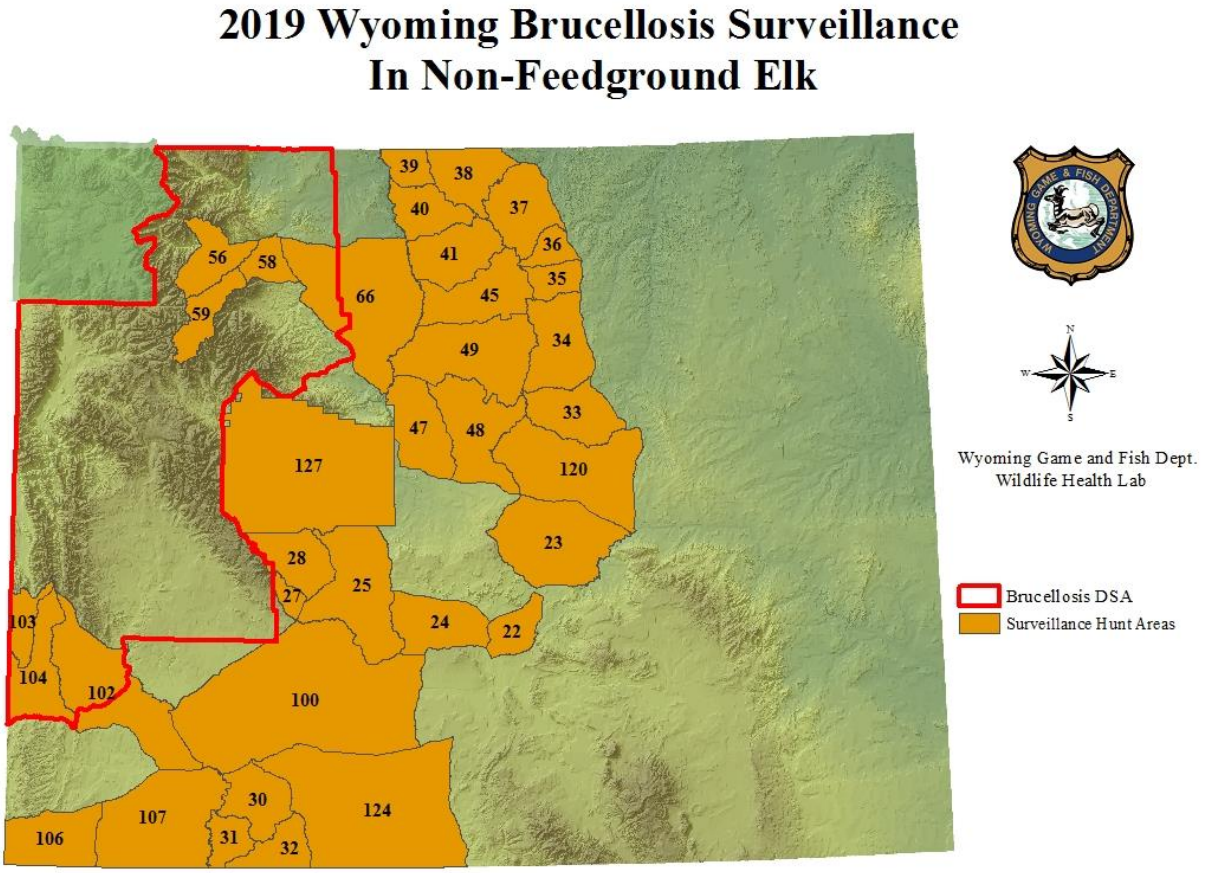

Figure 8. Elk hunt areas targeted for brucellosis surveillance in 2019.

Literature cited

Brennan, A., P. C. Cross, M. D. Higgs, W. H. Edwards, B. M. Scurlock, and S. Creel. 2014. A multi-scale assessment of animal aggregation patterns to understand increasing pathogen seroprevalence. Ecosphere 5(10):138. http://dx.doi.org/10.1890/ES14-00181.1